END
DATE
FILMED

84

DTIC

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963 A

INSTITUTE REPORT NO. 161

DOMESTIC SWINE IN PHYSIOLOGICAL RESEARCH
VI.   Effect of Splenic Erythrocyte Sequestration on Blood Volume Measurements
in Conscious Immature Pigs

JOHN P. HANNON, PhD
CAROL A. BOSSONE, BS, SP5
and
WILLIAM G. RODKEY, DVM, MAJ VC

DTIC
SELECTE
JAN 9   1984

A

NOVEMBER 1983

84   01   09   059

Domestic Swine in Physiological Research. VI. Effect of Splenic
Erythrocyte Sequestration on Blood Volume Measurements in Conscious
Immature Pigs--Hannon, Bossone, Rodkey

This material has been reviewed by Letterman Army Institute
of Research and there is no objection to its presentation and/
or publication. The opinions or assertions contained herein
are the private views of the author(s) and are not to be con-
strued as official or as reflecting the views of the Department
of the Army or the Department of Defense. (AR 360-5)

17 NW 83

(Signature and date)

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
LAIR INSTITUTE REPORT NO. 161	AD-A13668	

4. TITLE (and Subtitle)	5. TYPE OF REPORT & PERIOD COVERED
DOMESTIC SWINE IN PHYSIOLOGICAL RESEARCH VI. Effect of Splenic Erythrocyte Sequestration and Blood Volume Measurements in Conscious Immature Pigs	Interim May-Oct 1983
	6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)	8. CONTRACT OR GRANT NUMBER(s)
John P. Hannon, PhD Carol A. Bossone, BS William G. Rodkey, DVM	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
Division of Combat Casualty Care Letterman Army Institute of Research Presidio of San Francisco, CA 94129	Prog El 61102A, Task BA Project 3M161102BS10 Work Unit 256

11. CONTROLLING OFFICE NAME AND ADDRESS	12. REPORT DATE
US Army Medical Research & Development Command Ft. Detrick, Frederick, MD 21701	November 1983
	13. NUMBER OF PAGES 31

14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report)
	Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

This document has been approved for public release and sale; its distribution is unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Erythrocyte Volume; Blood Volume; Spleen; Swine

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

see reverse

20. ABSTRACT

When estimated by the dilution of ^{51}Cr-labelled erythrocytes under near-basal conditions, immature splenectomized pigs (N=20) had a circulating erythrocyte volume of 17.8+1.64 (S.D.) ml/kg. At an assumed body to large vessel hematocrit (BH:LH) ratio of 1.0 plasma volume was 42.8+2.69 ml/kg and blood volume 60.6+3.30 ml/kg. At an assumed BH:LH ratio of 0.9 plasma volume was 49.6+3.12 ml/kg and blood volume was 67.3+3.67 ml/kg. Sham-operated pigs (N=20) had a circulating erythrocyte volume of 16.2+1.39 ml/kg, and at a BH:LH ratio of 1.0 a plasma volume was 44.4+2.96 and a blood volume was 60.5+3.78 ml/kg; at BH:LH ratio of 0.9 plasma volume was 51.1+3.42 ml/kg and blood volume was 67.2+4.12 ml/kg. Kinetic analysis of early ^{51}Cr loss from the circulating blood of the sham-operated pigs indicated a splenic erythrocyte sequestration of 4.51+0.89 ml/kg and a t-1/2 of 9.76+1.93 minutes for splenic erythrocyte turnover. Epinephrine injection (0.025 mg/kg) caused rapid mobilization of splenic erythrocytes in sham-operated pigs (N=6) with a hematocrit rise from 0.27+0.01 to 0.35+0.02. Similar results were obtained in sham-operated pigs (N=9) subjected to physical restraint. Collectively, these measurements indicated that the porcine spleen, under near-basal conditions, sequestered 20-25 percent of the total body erythrocyte volume. Volume estimates in splenectomized pigs (N=7) based on simultaneous dilutions of ^{51}Cr-labelled erythrocytes and ^{125}I-labelled bovine albumin gave circulating erythrocyte, plasma and blood volumes of 18.4+2.46, 60.7+4.01 and 79.0+3.51 ml/kg, respectively, and a BH:LH ratio of 0.756+0.029. The latter value, it was concluded, reflected overestimation of plasma volume by the ^{125}I-labelled albumin procedure.

ABSTRACT

When estimated by the dilution of ^{51}Cr-labelled erythrocytes
under near-basal conditions, immature splenectomized pigs (N=20) had
a circulating erythrocyte volume of 17.8+1.64 (S.D.) ml/kg. At an
assumed body to large vessel hematocrit (BH:LH) ratio of 1.0 plasma
volume was 42.8+2.69 ml/kg and blood volume 60.6+3.30 ml/kg. At an
assumed BH:LH ratio of 0.9 plasma volume was 49.6+3.12 ml/kg and
blood volume was 67.3+3.67 ml/kg. Sham-operated pigs (N=20) had a
circulating erythrocyte volume of 16.2+1.39 ml/kg, and at a BH:LH
ratio of 1.0 a plasma volume was 44.4+2.96 and a blood volume was
60.5+3.78 ml/kg; at BH:LH ratio of 0.9 plasma volume was 51.1+3.42
ml/kg and blood volume was 67.2+4.12 ml/kg. Kinetic analysis of
early ^{51}Cr loss from the circulating blood of the sham-operated pigs
indicated a splenic erythrocyte sequestration of 4.51+0.89 ml/kg and
a t-1/2 of 9.76+1.93 minutes for splenic erythrocyte turnover.
Epinephrine injection (0.025 mg/kg) caused rapid mobilization of
splenic erythocytes in sham-operated pigs (N=6) with a hematocrit
rise from 0.27+0.01 to 0.35+0.02. Similar results were obtained in
sham-operated pigs (N=9) subjected to physical restraint.
Collectively, these measurements indicated that the porcine spleen,
under near-basal conditions, sequestered 20-25 percent of the total
body erythrocyte volume. Volume estimates in splenectomized pigs
(N=7) based on simultaneous dilutions of ^{51}Cr-labelled erythrocytes
and ^{125}I-labelled bovine albumin gave circulating erythrocyte,
plasma and blood volumes of 18.4+2.46, 60.7+4.01 and 79.0+3.51
ml/kg, respectively, and a BH:LH ratio of 0.756+0.029. The latter
value, it was concluded, reflected overestimation of plasma volume
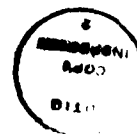by the ^{125}I-labelled albumin procedure.

Key Words: erythrocyte volume, blood volume, spleen, swine.

i

PREFACE

Previous Institute Reports, Letterman Army Institute of Research, on Domestic Swine in Physiological Research have included the following titles:

I.   A Biomedical Model. Report No. 91, May 1981

II.   Electrolyte Values for Arterial Serum from Young Anesthetized Pigs Maintained under Steady-State Ventilatory Conditions. Report No. 92, May 1981

III.   Blood Gas and Acid-Base Values for Arterial Blood from Young Anesthetized Pigs Maintained under Steady-State Conditions. Report No. 113, February 1982

IV.   A Blood Acid-Base Curve Nomogram for Immature Pigs. Report No. 137, February 1983

V.   Construction of Acid-Base Alignment Nomograms to Estimate Buffer Base and Base Excess Concentrations in Arterial Blood from Immature Pigs. Report No. 144, April 1983

ACKNOWLEDGMENTS

TABLE OF CONTENTS

DOMESTIC SWINE IN PHYSIOLOGICAL RESEARCH
VI. EFFECT OF SPLENIC ERYTHROCYTE SEQUESTRATION ON BLOOD VOLUME
MEASUREMENTS IN CONSCIOUS IMMATURE PIGS

Reports on porcine blood volume measurement show a wide range of
values for supposedly normal animals. Much of this variability is
attributable to differences in body size, an effect first reported in
1951 by Hansard et al (1). In a limited number of animals these
investigators (1) showed by means of ^{32}P-labelled erythrocyte dilution
that blood volume per kilogram decreased as body weight increased. At
one extreme, in 10-kg pigs, a value of 74 ml/kg was obtained while at
the other extreme, in 344-kg pigs, a value of 45 ml/kg was
obtained (1). Similar size-related decrements in porcine blood volume
have been reported subsequently by a number of others. The available
data, through 1972, have been assembled and reviewed by Steinhardt et
al (2). Regression equations predicting blood volume as a function of
body weight have been reported by von Engelhardt (3).

Decrements in blood volume with increasing body size are largely
attributable to increases in the fat fraction of total body
mass (4-6). However, widely divergent values have been reported also
for plasma and erythrocyte volumes in pigs of similar body weight (2).
Some of this divergency appears due to the particular indicator
dilution procedure that was used in the volume estimates. Procedures
based on the dilution of Evans Blue or radioiodine-labelled serum
albumin produce generally higher volume estimates than procedures
based on the dilution of ^{32}P-or ^{51}Cr-labelled erythrocytes.
Additional variability, in the case of albumin labels, may be caused
by the use of different techniques to correct for transcapillary
albumin losses following indicator injection.

One variable that has been largely ignored in porcine blood
volume measurements is potential sequestration, or discharge, of
erythrocytes from the spleen during the measurement period. The
porcine spleen is a contractile organ (7,8) which can release large
numbers of erythrocytes and markedly elevate hematocrit levels when
stimulated by epinephrine (7,8) or exercise (8). Steinhardt and his
coworkers (8-11) have attempted to measure the impact of such splenic
erythrocyte mobilization on porcine blood volume estimated by Evans
Blue dilution. These attempts, however, have been less than
satisfactory because the measurements did not address potential
alterations in transcapillary indicator loss. In the dog at least,
splenic sequestration or discharge of erythrocytes has a major effect
on blood volume (12,13).

In the study described here erythrocyte storage capacity of the porcine spleen was assessed by kinetic analysis of ^{51}Cr-labelled erythrocyte dilution following intraarterial injection into conscious pigs. Storage capacity also was assessed by changes in circulating erythrocyte volume subsequent to physical restraint of the animal and by hematocrit changes subsequent to epinephrine injection. In addition, blood volume characteristics were compared in sham-operated and splenectomized pigs. Finally, vascular volumes based on the dilution of ^{51}Cr-labelled erythrocytes were compared to those based on the dilution of ^{125}I-labelled bovine serum albumin.

METHODS

Fifty-seven immature Yorkshire pigs, both barrows and gilts, were obtained from a commercial breeder (J.G. Boswell Co., Corcoran, CA) and were maintained in a common indoor holding area at Letterman Army Institute of Research until utilized for study, usually within two to four weeks after arrival. They were fed a commercial ration (Purina Pig Chow, Ralston Purina Co., St Louis, Mo) and water ad libitum. The pigs were 2 to 3 months old and weighed (mean $\pm$ S.D.) 22.6 $\pm$ 2.21 kg when the studies were conducted.

After an overnight fast, each pig received a pre-anesthetic intramuscular injection of 2.2 mg/kg atropine sulfate 2.2 mg/kg ketamine HCl, and 2.2 mg/kg xylazine. Halothane anesthesia was induced by face mask and maintained with an endotracheal catheter. A celiotomy was performed and, in 31 animals, the spleen was removed according to standard techniques with double ligation of all vascular pedicles. All celiotomies were closed with two layers of non-absorbable surgical suture. The remaining 26 animals were sham operated, that is, abdominal incisions were made, then closed immediately.

The left carotid artery of each pig was exposed and a 1.27-mm ID x 2.03-mm OD polyvinylidine catheter (Type S-54 HL Tygon, Norton Plastics and Synthetics, Akron, OH) was inserted to the level of the aorta and secured by ligatures around the carotid artery. The free end of the catheter was tunneled beneath the skin and exited on the dorsal surface of the neck. The exteriorized portion was fitted with a 17-gauge Intramedic^R Luer Stub Adapter (Clay Adams, Parsippany, N.J.) and capped with an Argyle^R Intermittent Infusion Plug (Brunswick Co., St Louis, Mo). Following wound closure the catheter was filled through the infusion plug with heparin (1000 units/ml). The catheter exit site was protected by a 5-cm x 10-cm Velcro^R (Velcro USA, New York, NY) patch sutured to the skin; a 2-cm x 7.5-cm hole was cut in the patch portion next to the skin to allow catheter access.

After a 7- to 10-day recovery period each pig, after an overnight fast, was brought into a quiet laboratory in a portable transport cage

and given a variety of fabric bedding material. After 15 to 30
minutes of rooting and bedding rearrangement, most animals voluntarily
assumed a recumbent position. When so positioned, the intermittent
infusion plug was removed and the stub adapter was connected to a 12-
inch pressure monitoring/injection line (Cobe Laboratories, Lakewood,
Co). The latter had been previously fitted with a plastic three-way
stopcock (Pharmaseal[R], Inc., Toa Alta, Puerto Rico) and filled with
heparinized saline (10 units/ml). The entire system was then cleansed
by withdrawing 10 ml of fluid (blood plus heparinized saline) followed
by flushing with fresh heparinized saline. After 15 to 30 minutes of
additional voluntary recumbent rest, the experiment was started.
Three experiments were conducted.

Experiment 1: Determination of Circulating and Total Body Erythrocyte
Volume

Eight splenectomized and 8 sham-operated pigs were used to
ascertain time-related changes in concentration of arterially injected
[51]Cr-tagged erythrocytes. Twenty-five milliliters of blood were
withdrawn from each animal and mixed with 4 ml of ACD solution and
approximately 50 uCi $Na_2{}^{51}Cr O_4$. After standing for 30 minutes at
room temperature, with occasional mixing, the blood was centrifuged at
$4^{\circ}C$ and 2500 g for 10 minutes, and the plasma was discarded. The
erythrocytes were then washed twice by resuspension in cold isotonic
saline followed by centrifugation. After the last wash, the
erythrocytes were thoroughly mixed with 10 ml of cold saline,
preparatory to injection into the pig; final volume, including
erythrocytes, was approximately 13 ml. A calibrated syringe was used
to inject 10 ml of the mixture rapidly into the pig. This maneuver
was accomplished by means of a rubber injection bulb and pressure
monitoring line attached to the carotid catheter. The injectate was
flushed in with 20 ml of saline, the entire procedure required about
10 seconds. In vivo dilution of ^{51}Cr-tagged erythrocytes was
determined in blood samples (3 ml) taken at 1-minute intervals over
the first 10 minutes, and at 15, 20, 30, 45, 60, 90, 120 and 150
minutes after injection. To assure the acquisition of fresh
circulating blood, sample removal was immediately preceded by a
15-second period of continuous blood withdrawal from the catheter at a
rate of 1 ml/sec. After sample removal, this blood was returned to
the animal, and the catheter was filled with a minimal volume, about 1
ml, of heparinized saline (10 units/ml).

A Packard Auto-gamma counter, Model 5986, (Packard Instrument Co,
Downers Grove, Ill), with an energy window set at 276 to 366 KEV, was
used to count ^{51}Cr-tagged erythrocyte activity. A 1-ml aliquot of
each blood sample and triplicate 0.2-ml aliquots of the erythrocyte-
saline injected dose were transferred to 12 x 75 mm disposable test

tubes and diluted with water to 2.0 ml preparatory to counting. Positive displacement micropipettes (Scientific Manufacturing Industries, Emeryville, CA) were used for transfers of the blood sample and injected dose. The hematocrit of each blood sample was determined with a Lourdes microhematocrit centrifuge (Vernitron Medical Products, Inc., Carlstadt, NJ), and the ^{51}Cr activity per milliliter of erythrocytes, corrected for trapped plasma, was calculated.

The foregoing data were supplemented by similar data collected from an additional 12 splenectomized and 12 sham-operated pigs to describe porcine population characteristics for circulating erythrocyte volume, total body erythrocyte volume, splenic (in the case of sham-operated pigs) erythrocyte volume, circulating plasma volume, circulating blood volume, and the kinetic characteristics of splenic erythrocyte exchange with the systemic circulation. The dilution of ^{51}Cr-tagged erythrocytes in these additional pigs was measured at 5,10,15,20,30,45,60, and 90 minutes after injection. In splenectomized pigs, the injected dose was divided by the average ^{51}Cr CPM/ml of erythrocytes to obtain the circulating and total body erythrocyte volumes.

Determinations of circulating and total body erythrocyte volume in sham-operated pigs was complicated by splenic sequestration of ^{51}Cr-tagged erythrocytes. Such sequestration and release of untagged previously stored erythrocytes caused a rapid decline in the circulating dilution values. This decline showed first-order kinetics with an asymptotic value being approached 30 to 150 minutes after injection. The general equation describing dilution of ^{51}Cr-tagged erythrocytes in these animals would be:

$$A_t = A_m + a_0 e^{-kt} \qquad [1]$$

where A_t equals ^{51}Cr activity at any time t after injection, A_m equals the asymptotic value for complete mixing of ^{51}Cr-tagged erythrocytes in the total body erythrocyte volume, a_0 is the zero-time intercept and k is the rate constant for the fraction of circulating erythrocytes entering or leaving the spleen per unit time. A least-squares-regression procedure, employing iterations of A_m estimates, is used to calculate a_0 and k. Thus, an approximate A_m value is chosen, for example the ^{51}Cr-tagged erythrocyte CPM observed 30 minutes after injection, and this value is subtracted from all of the values recorded previously. The resultant differences are entered into Equation 1 along with their respective t values, and a_0, k, the correlation coefficient, r, and the variance are calculated. This procedure is repeated with revised estimates of A_m until maximum r and minimum variance values are obtained. At this point the injected dose divided by A_m would equal the total body erythrocyte volume. The injected dose divided by $A_m + a_0$ would equal the circulating erythrocyte volume, and the difference between total body and

circulating erythrocyte volumes would equal the volume of erythrocytes
sequestered in the spleen. In addition, the   value can be used to
determine the half-time of splenic erythrocyte turnover. Calculation
of A_m, A_0 and k values can be a time-consuming
procedure when done by hand, but fortunately readily available
computer programs expedite the task.

In both sham-operated and splenectomized pigs arterial hematocrit
values were used to estimate circulating plasma and blood volumes.
Two procedures were used. In one, equality of arterial and total body
hematocrit values was assumed. Thus,

$$PV = EV\ \frac{1 - (0.97)(Hct)}{(0.97)(Hct)} \qquad [2]$$

where PV and EV represent circulating plasma and erythrocyte volumes,
respectively, Hct represents the arterial hematocrit and 0.97 is a
correction factor for trapped plasma. In the second procedure, the
total body hematocrit value was assumed to be 90 percent of the
arterial hematocrit value. Thus,

$$PV = EV\left[\frac{1 - (0.9)(0.97)(Hct)}{(0.9)(0.97)(Hct)}\right] \qquad [3]$$

With either procedure, the sum of circulating erythrocyte and plasma
volumes was used to estimate circulating blood volume.

Experiment 2: Measurement of Splenic Erythrocyte Mobilization

Two procedures were used to assess porcine capacity for
circulatory mobilization of erythrocytes sequestered in the spleen.
In one, control values for hematocrit, and circulating erythrocyte,
plasma and blood volumes were measured with ^{51}Cr-tagged erythrocytes
in 8 splenectomized and 8 sham-operated pigs under conditions of
voluntary recumbent rest. These measurements were obtained during the
course of the Experiment One. Immediately after the last blood sample
was withdrawn in the first experiment the recumbent pig was grabbed by
the front legs, raised to a vertical position and placed on its
buttocks. This led to considerable agitation and struggle on the part
of the animal, but it was physically restrained in such a position for
one minute. Then, an additional 3-ml blood sample was withdrawn for
hematocrit determination, and ^{51}Cr counting, from which the changes in
circulating erythrocyte, plasma and blood volumes, relative to earlier
control values, were calculated; body to venous hematocrit ratios of
0.9 and 1.0 were assumed.

In the other procedure, a series of 4 control hematocrit values
were determined at 5 minute intervals in 4 splenectomized and 6 sham-
operated pigs not used in the other experiments. Again, the pigs

remained in a voluntary recumbent position while control blood samples
were obtained. After the last sample was removed the pig was injected
intraarterially with 0.5 ml of epinephrine (1 mg/ml), and blood
samples for hematocrit determination were taken at 2.5,5,10,20,40, and
80 minutes after injection. Epinephrine caused an immediate arousal
of the animal, but in all instances recumbency was resumed after 10 or
15 minutes. The changes in hematocrit following epinephrine were used
as an index of splenic erythrocyte mobilization.

Experiment 3:   Simultaneous Determination of Circulating Erythrocyte
                and Plasma Volumes

     The erythrocyte volume of 7 splenectomized pigs was estimated by
the dilution of ^{51}Cr-tagged erythrocytes, as in Experiment One. In
addition, plasma volume was estimated stimulaneously by the dilution
of ^{125}I-tagged bovine serum albumin. In the latter instance,
approximately 10 uCi of ^{125}I-albumin was diluted in 15 ml of the
animal's plasma and 10 ml was injected through the carotid artery
catheter and flushed in with 20 ml of saline. The entire procedure
required about 10 seconds. Samples for erythrocyte ^{51}Cr and plasma
^{125}I counting were withdrawn, as in Experiment One, at 1-minute
intervals for the first 10 minutes, and at 15,20,30,45,60,90,120 and
150 minutes after injection. Plasma was collected following sample
centrifugation and 1-ml aliquots, obtained with a positive
displacement pipette, were counted in the Model 5986, Packard Auto-
gamma counter at an energy window of 18 to 80 KEV. Radioactivity in
the injected dose was determined by triplicate counts of 0.2-ml
aliquots obtained with a positive displacement micropipette. A
semilogarithmic, least-squares extrapolation of plasma ^{125}I
concentration between 30 and 150 minutes after injection was used to
calculate the zero-time plasma ^{125}I dilution and this value in turn
was used to calculate plasma volume. Blood volume was calculated as
the sum of ^{51}Cr-determined erythrocyte and ^{125}I-determined plasma
volumes. Blood volume so measured was divided into ^{51}Cr-determined
erythrocyte volume to obtain the total body hematocrit value, and then
this value was divided by the arterial hematocrit to obtain the total
body to large vessel hematocrit ratio. Finally, the arterial
hematocrit value and an assumed total body to large vessel ratio of
1.0 were used to calculate plasma and blood volumes from ^{51}Cr-
determined erythrocyte volume, and erythrocyte and blood volume from
^{125}I-determined plasma volume.

     Accuracy of the pipettes used in this study was assessed
gravimetrically first with distilled water and subsequently with
blood. In the latter instance, blood density was first obtained by
weighing exactly 100 ml in a volumetric flask and using the same blood
to determine the blood weight, and calculated volume, delivered by the

0.2-ml and 1.0-ml positive displacement pipettes and the 10 ml calibrated injection syringes.

In addition, hematocrit values used in the calculation of erythrocyte and plasma volumes were corrected for trapped plasma, as determined by a procedure similar to that reported by Baker (14). Briefly, an accurate, gravimetrically measured volume (30 to 70 ml) of porcine blood was placed in a beaker along with an accurately measured dose of ^{125}I-bovine albumin or $Na_2^{35}SO_4$. The blood and isotope were gently, but thoroughly, mixed for 10 minutes with a magnetic stirrer, at the end of which 5 or more microhematocrit capillary tubes were filled and centrifuged at 12,000 g for 5 minutes. The remaining blood was centrifuged at 2500 g for 10 minutes, plasma was collected and the activity of ^{125}I-bovine albumin or $Na_2^{35}SO_4$ was determined in 5 or more 1-ml plasma aliquots. Average isotope dilution was used to calculate the beaker plasma volume, and, by difference from the original beaker blood volume, the beaker erythrocyte volume and hematocrit. Finally, the difference between beaker hematocrit and the slightly lower average capillary tube hematocrit was used to calculate the plasma fraction trapped in the erythrocyte column packed by the microhematocrit centrifuge.

RESULTS

As indicated in Table 1, a high degree of volume measurement accuracy was obtained with the positive displacement pipettes and injection syringes. No significant differences were observed between the volume of water and blood delivered by each type of the pipette or syringe.

Trapped plasma contained in the packed erythrocyte column following microhematocrit centrifugation ranged from 1.2 to 5.0% (Table 2). There was no significant difference between the percentage estimated by ^{125}I dilution and the percentage estimated by ^{35}S dilution. The composite average was 2.8 ± 1.26% (mean ± S.D.).

Experiment 1: Circulating and Total Body Erythrocyte Volume

Figure 1 shows the normalized dilution characteristics of ^{51}Cr-tagged erythrocytes over the first 45 minutes after injection in sham-operated and splenectomized pigs; the data were normalized by assigning a value of 100 to the extrapolated zero-time dilution value. Beyond 45 minutes, values in the splenectomized pigs showed no statistically significant change from those obtained several minutes after injection. Values obtained 1 or 2 minutes after injection tended to be higher than those recorded subsequently. These apparent elevations, and suggestions of cycling over the first 10 minutes after injection, may have been due to recirculation of blood through vascular circuits close to the point of injection and sampling.

Table 1. Measurement accuracy of 0.2 and 1.0 ml positive displacement sample transfer pipettes and 10.0 ml calibrated injection syringes

	0.2 ml		1.0 ml		10 ml	
Trial*	H_2O	Blood	H_2O	Blood	H_2O	Blood
1	0.1989	0.2000	0.9951	0.9993	9.9915	9.9906
2	0.1996	0.1968	1.0040	1.0033	10.0078	10.0253
3	0.2002	0.1974	0.9964	1.0004	10.0041	9.9990
4	0.1994	0.1999	0.9978	0.9930	9.9935	9.9836
5	0.1993	0.1993	1.0048	0.9968	9.9985	10.0070
Mean	0.1995	0.1987	0.9996	0.9986	9.9991	10.0011
S.D.	+0.00048	+0.00066	+0.00200	+0.00174	+0.00308	+0.00722

* Each trial value, in ml, represents the average of triplicate measurements of distilled water or blood. Replacement 0.2 and 1.0 plastic tips and different 10 ml syringes were used for each trial.

Table 2. Trapped plasma (T.P.) as determined by the ^{125}I-albumin or Na_2 [35]SO_4 contained in the erythrocyte column packed by microhematocrit centrifugation

	^{125}I-albumin			Na_2 [35]SO_4
Trial*	Hct	T.P.(%)	Hct	T.P.(%)
1	0.22	3.2	0.26	2.0
2	0.28	2.7	0.33	2.2
3	0.29	2.3	0.30	4.7
4	0.30	1.2	0.28	1.2
5	0.30	2.1	0.33	4.1
6	0.29	5.0	0.29	3.4
Mean	0.28	2.7	0.30	2.9
S.D.	+0.030	+1.28	+0.028	+1.35

*Blood from separate pigs (N=12) was used in each trial.
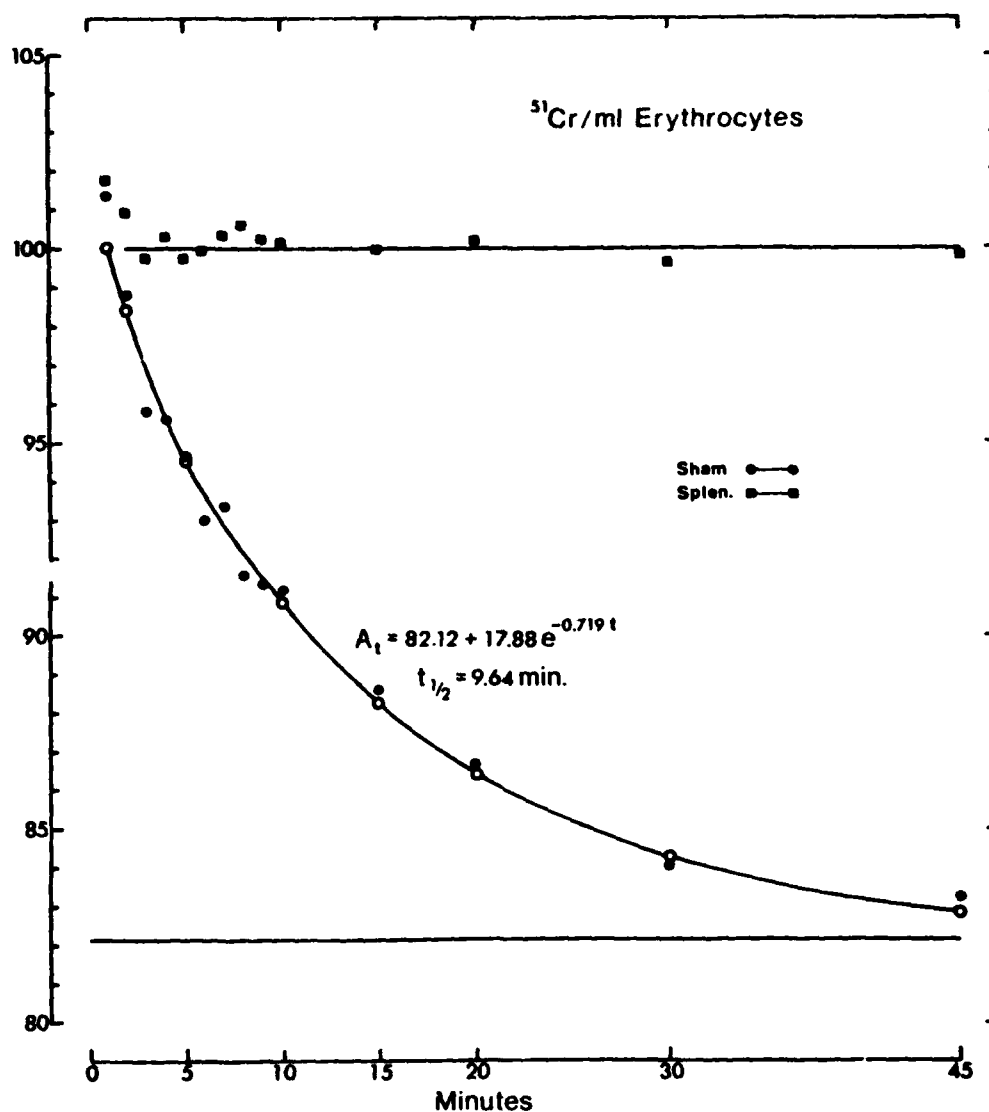
Figure 1. Alterations in the concentration of ^{51}Cr-tagged erythrocytes as a function of time after injection. Upper horizontal line indicates average value in splenectomized pigs (N=8). Lower horizontal line indicates asymptotic value reflecting complete body mixing in sham-operated pigs (N=8); open circles indicated points calculated by the indicated regression equation. Ordinate values are normalized such that concentration at t=0 is assigned a value of 100.

Splenectomized pigs had slightly but significantly higher hematocrit values and circulating erythrocyte volumes and slightly lower-circulating plasma volumes compared to the values found in sham-operated pigs (Table 3). Total circulating blood volume was essentially equal in both groups. Sham-operated pigs had a calculated splenic erythrocyte volume of 4.51 ± 0.89 (S.D.) ml/kg which represented about 22% of the total body erythrocyte volume.

Table 3. Blood volume characteristics of sham-operated and splenec-tomized pigs as estimated by ^{51}Cr-labelled erythrocytes

Measurement		Sham-operated Pigs	Splenectomized Pigs
		N = 20	N = 20
Hematocrit		0.267 ± 0.0153	0.293 ± 0.022*
Circ Erythro		16.16 ± 1.386	17.76 ± 1.644*
Total Erythro Vol		20.67 ± 1.658	17.76 ± 1.644*
Splenic Erythro Vol		4.51 ± 0.894	0
Circ Plasma Vol	(A)	44.33 ± 2.964	42.85 ± 2.694*
Circ Plasma Vol	(B)	51.09 ± 3.416	49.59 ± 3.118*
Circ Blood Vol	(A)	60.48 ± 3.776	60.60 ± 3.301
Circ Blood Vol	(B)	67.24 ± 4.198	67.34 ± 3.668

All volume measurements expressed as mean ml/kg ± S.D.
Volume calculations after (A) assume a total body to large vessel hematocrit ratio of 1.0, after (B) a ratio of 0.9.

*Significant (P<0.05) difference between groups.

Because of this splenic component, total body erythrocyte volume was significantly greater in sham-operated than in splenectomized pigs. The average t 1/2 value for splenic red cell turnover in 20 pigs was 9.76 ± 1.93 minutes. Applicaton of regression Equations 1 for ^{51}Cr-labelled erythrocyte concentration in individual pigs showed that values of a_0, as anticipated, varied in direct proportion to the injected dose and / . More specifically a_0, on the average, equaled $0.213 A_m$.

The following average equation, therefore, reflected the population characteristics of the sham-operated immature domestic pigs used in this study:

$$A_t = A_m (1 + 0.213 e^{-0.739 t})$$ [4]

Experiment 2: Splenic Erythrocyte Mobilization

Table 4 summarizes the effects of 1-minute of physical restraint, subsequent to a control period of recumbent rest, on the circulating blood volume and related variables of sham-operated and splenectomized pigs. In both groups, physical restraint was associated with a significant increase in hematocrit, but the effect was significantly (by analysis of variance) more pronounced in the sham-operated animals. This difference, reflecting splenic erythrocyte mobilization, caused the sham-operated animals to have elevated circulating erythrocyte volumes following physical restraint. In both groups restraint led to comparable decrements in calculated circulating plasma volume which in the splenectomized pigs led to a significant decrease in calculated blood volume. Physical restraint had no effect on the blood volume of sham-operated pigs; the decrement in plasma volume was offset by splenic erythrocyte mobilization.

Table 4. Effects of physical restraint on the blood volume character-istics of sham-operated and splenectomized pigs

Measurement	Sham-Operated Pigs		Splenectomized Pigs	
	Control	Restrained	Control	Restrained
Hematocrit	0.269+0.078	0.347+0.013*	0.281+0.021	0.302+0.022*
51Cr/ml Erythro	17.2+3.54	17.1+3.42	17.3+2.72	17.4+2.77
Circ Erythro Vol	16.3+0.88	21.1+1.70*	17.1+1.61	17.1+1.63
Circ Plasma Vol(A)	44.1+2.68	39.7+0.86*	43.8+3.01	36.4+2.57*
Circ Plasma Vol(B)	51.0+3.10	46.5+1.00*	50.5+3.47	45.8+3.23*
Circ Blood Vol(A)	60.4+3.34	60.8+3.81	60.9+3.69	56.4+3.26*
Circ Blood Vol(B)	67.3+3.72	67.6+4.24	67.6+4.10	62.9+3.64*

All measurements expressed as mean ml/kg $\pm$ S.D. for 8 sham-operated and 8 splenectomized pigs. ^{51}Cr C.P.M. expessed in thousands. Volume calculations after (A) assume a total body to large vessel hematocrit ratio of 1.0, after (B) a ratio of 0.9.

*Indicates a significant (P<0.05) difference between control and restained values.

Similar hematocrit effects were observed when resting sham-operated and splenectomized pigs received intraarterial injections of epinephrine (Fig 2). No attempt was made to assess the effects of epinephrine on vascular volumes because the rate and extent of splenic erythrocyte storage during the recovery phase could not be estimated in the sham-operated pigs. Nevertheless, the hematocrit increments observed immediately afer injection were nearly identical to those observed after physical restraint.
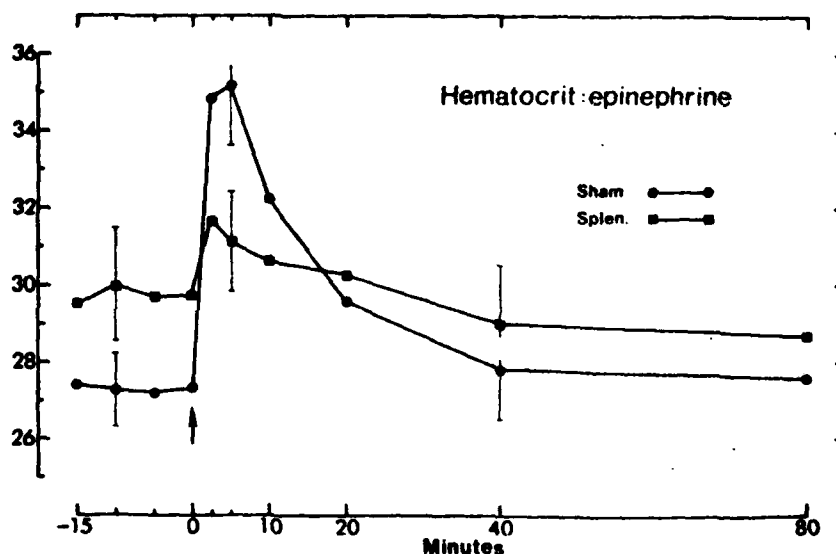

Figure 2. Effect of a 0.5 ml, 1:1000 dilution epinephrine injection (arrow) on the hematocrit of splenectomized (N=4) and sham-operated (N=6) conscious pigs.

Experiment 3: Simultaneous Erythrocyte and Plasma Volume M asurements

Attempts to measure circulating plasma volume by the dilution of ^{125}I-labelled bovine albumin dilution were complicated by disappearance of the label from the circulation. Kinetic evaluation of this disappearance revealed two components, suggesting at least two pools of albumin dilution (Fig 3). The larger pool which became apparent about 20 minutes after injection had a t 1/2 of 6.26 hours; the smaller pool which became apparent about 2 minutes after injection had a t 1/2 of 4.87 minutes. Plasma volume estimates were based on least-squares extrapolation of the larger pool to zero time as indicated in Figure 3. The blood volume characteristics of splenectomized pigs as estimated by the dilution of ^{51}Cr-labelled erythrocytes and ^{125}I-labelled albumin are summarized in Table 5. These data show that
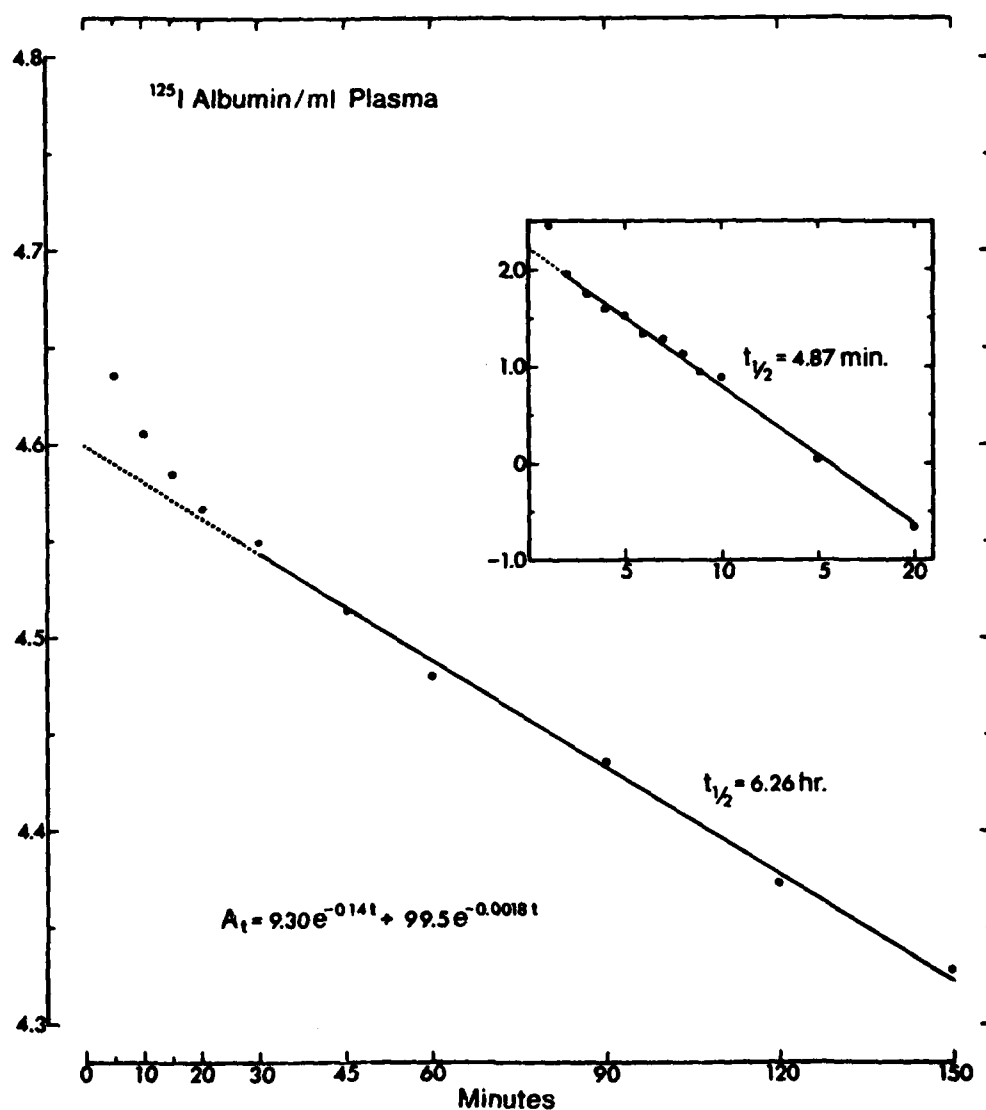
Figure 3. Alterations in the plasma concentration ^{125}I-tagged albumin as a function of time in conscious splenctomized pigs (N=7). Ordinate values expressed as the natural logarithm of the normalized ^{125}I activity, a value of 100 being assigned to the activity recorded 10 minutes after injection.

^{125}I-tag ed albumin dilution gives a significantly greater plasma,
erythr and blood volume than ^{51}Cr-tagged erythrocyte dilution.
As wou. expected, blood volumes determinations based on the two
radiolabels were intermediate to those obtained by either label alone.
The average body hematocrit was considerably lower than the arterial
hematocrit, the ratio of the two being 0.756 ± 0.029.

Table 5. Porcine blood volume characteristics as estimated by the
dilution of ^{51}Cr-labelled erythrocytes and ^{125}I-labelled
plasma albumin

Measurement	^{51}Cr-Erythro. Dilution	^{125}I-Albumin Dilution	Diff.
Erythrocyte Volume	18.4±2.46	27.0±3.62	8.6*
Plasma Volume	41.3±3.33	60.7±4.04	19.4*
Blood Volume	59.7±3.43	87.7±4.46	28.0*
^{51}Cr/^{125}I Blood Volume	79.0±3.51		
Body Hematocrit	0.233±0.024		
Arterial Hematocrit	0.308±0.022		
Body/Arterial Hct Ratio	0.756±0.029		

Indicated values represent the mean ml/kg ± S.D. for 7 animals

*Significant (P<0.05) difference.

DISCUSSION

The results of this study show that the spleen of the immature,
near-basal pig sequesters 20 to 25 percent of the total body
erythrocyte volume. Under these conditions, the turnover of
sequestered splenic erythrocytes averages about 7 percent per minute.
Under conditions other than basal, however, splenic contraction can
cause a rapid transfer of stored cells to the circulating blood. In
the present experiments, such erythrocyte mobilization was observed
following the intraarterial injection of epinephrine or following a
brief period of physical restraint which, presumably, led to
endogenous catecholamine release. Splenic contraction and erythrocyte
release, as evidenced by increased venous hematocrit levels, has been
studied extensively by Steinhardt and his coworkers (2,8,10,11) in
domestic pigs subjected to treadmill exercise or to intravenous
epinephrine injections. Epinephrine-induced splenic contraction in
minipigs has also been investigated by Wachtel and McCahan (7).

Interpretational difficulties are encountered when the results of
these studies are compared to those recorded here, largely because
experimental conditions associated with data collection were not
equivalent. For instance, Steinhardt et al (8) investigated the
hematocrit responses of intact and splenectomized pigs intravenously
injected with epinephrine (0.008 mg/kg) while the animals were
restrained in a lateral recumbent position. Not only was their dosage
of epinephrine lower than that used in the present study (about
0.025 mg/kg) but also their use of physical restraint could have
produced elevated control hematocrit values. Either of these factors
could explain the lower epinephrine-induced hematocrit increments
reported by Steinhardt et al (8), relative to those observed here.

Failure to recognize, or adequately account for, the erythrocyte
storage and autotransfusion functions of the porcine spleen also would
seem to be a major factor contributing to the wide variations in the
hematocrit values and circulating erythrocyte volumes reported for
normal pigs. In animals of the same general body size as those used
in the present study, normal hematocrit values range from about 0.30
to about 0.39 (15-18). In all of these reports (15-18), hematocrits
were measured in venous blood samples obtained by vena cava puncture
while the animals were being physically restrained. They are
substantially higher than those recorded here for arterial blood taken
from pigs under near-basal conditions but are similar to those
obtained after one minute of physical restraint. Splenectomy also
induces a slight elevation in resting hematocrit level, as seen in
Tables 3 and 4, and in data reported by Steinhardt et al (8) and by
Wachtel and McCahan (7). These increments may be due to splenic
contraction during the course of the splenectomy procedure itself. In
support of this contention, arterial and splenic venous hematocrit
values measured in 10 pigs during the early stages of spleen removal
averaged 0.28 ± 0.024 and 0.50 ± 0.110, respectively. Alternatively, the
splenectomized pigs may have responded to the lack of erythrocyte
reserves by increased erythropoietic activity.

In the intact pig, splenic blood flow and erythrocyte
sequestration can lead to overestimated values for circulating
erythrocyte volume when the measurements are based on the dilution of
labelled erythrocytes. Thus, following injection, the spleen acts as
a "sink" sequestering the injected cells and replacing them, via
splenic venous outflow, with endogenous unlabelled cells. This
process leads to a rapid decline in labelled erythrocyte concentration
shortly after injection but continues for an hour or more before the
ratio of labelled to unlabelled cells becomes equal in the circulating
blood and spleen. The sequestration-dilution effect seen here in pigs
(Figure 1) has been observed also in dogs (13,19) and cats (20).
Unless appropriate corrections are made, it will lead to an apparent
increase in calculated volume of circulating erythrocytes as a
function of time after injection. One-time measurement of label
dilution, therefore, usually produces an erroneous over-estimation of

circulating erythrocyte volume, the magnitude being dependent upon the
time after injection at which dilution is determined. Extrapolation
of dilution values to zero time will minimize or eliminate such
errors, but only if the extrapolation procedure is based on a two-
compartment model which segregates total body erythrocyte volume into
circulating and splenic components. Extrapolation is rarely, if ever,
attempted, possibly because of the difficulties encountered in
obtaining an adequate series of dilution samples shortly after
labelled erythrocyte injection. Inadequate or no correction for
splenic erythrocyte sequestration may have compromised measurement
accuracy in many studies (1,4,5,21-27) in which circulating
erythrocyte volume of swine was determined by the dilution of labelled
cells. Accordingly, reported values for pigs of comparable size
invariably exceed the circulating values, but approximate the total
body values seen here in conscious near-basal pigs. In some
instances (5,21,22) splenic discharge of sequestered erythrocytes,
induced by the use of physical restraint during the course of
measurement, could have elevated the values.

Indicator loss, in this instance to the interstitial fluid, can
also present problems when circulating plasma volume is estimated by
the dilution of serum albumin labelled with radioisotopes or T-1824
dye. Some (2,8-11,24,25,28,29) but not all (1,6,21-23,26,27,30-32) of
the investigators who have used these labels in swine have based their
volume estimates on the extrapolation of dilution values to zero time,
as was done in the present study with ^{125}I-labelled bovine serum
albumin. With albumin labels, one-time dilution samples do not appear
to alter seriously the magnitude of plasma volume estimates, provided
they are taken within 10 or 15 minutes after injection. The
radioisotope concentration in such samples (Figure 3) approximates the
extrapolated zero-time values. Either procedure, therefore, yields
about the same plasma volume value, and in pigs of comparable size
reported values (24,25,28) approximate those observed here.

The present study does raise serious questions about the validity
of plasma volume measurements based on the dilution of labelled
albumin. Plasma volumes so obtained were markedly greater than those
based on labelled erythrocyte dilution and large vessel hematocrit.
The plasma volume estimates, furthermore, produced elevated values for
circulating erythrocyte and blood volume when the latter are
calculated on the basis of large vessel hematocrits. In this respect,
similar values have been obtained by others in pigs of equivalent
size (23-25,28). These elevated plasma volume values also produced
elevated blood volume values when the erythrocyte component of blood
volume was determined simultaneously by the dilution of cells labelled
with ^{51}Cr. The elevated plasma volume values, however, led to a
calculated total body hematocrit which was considerably lower than
arterial hematocrit. In the present experiment with splenectomized
pigs, the ratio of the two averaged 0.756--a value slightly greater
than those reported in 6-week-old pigs by Talbot and Swenson (24) and

lower than the values for newborn pigs by Deavers et al (26) and Linderkamp et al (27) and for newborn to 12-week-old pigs by Setiabudi et al (25). Autotransfusion of splenic erythrocytes, and a resultant increase in the erythrocyte volume component, may have been responsible for the higher ratio values reported by some of these workers (25).

Total body to large vessel hematocrit ratios below 1.0 are commonly attributed to relative plasma excess in small vessels, including arterioles, capillaries, and venules (12,14,32). Indeed, direct estimation of small vessel hematocrits has yielded values ranging down to 0.1 or lower (33). In humans and dogs such reduced values in small vessels potentially could account for total body to large vessel hematocrit ratios of about 0.9, a commonly calculated value (12,32). In pigs, however, the ratio observed here does not seem compatible with this explanation. Thus, the contributions of small and large vessel hematocrits to total body hematocrit can be expressed by the following equation:

$$H_B = H_S + H_L(1 - V_S) \qquad [5]$$

where H_B, H_S, and H_L are the total body, average small vessel, and large vessel hematocrits, respectively, and V_S is the small vessel fraction of total blood volume. When H_L and H_B are measured, as in the present experiment with splenectomized pigs, a curve relating to various values of H_S can be constructed. Figure 4 shows the results of such an effort in which the H_L and H_B values recorded here in pigs are compared to commonly reported H_L and H_B values for humans (32). In pigs, but not in humans, these curves show that one would have to assume unreasonably large values for V_S to account for observed H_L and H_B values. For example, if the average H_S is assumed to be 0.1 (Fig. 4), then V_S would be 0.125, a reasonable value, in humans but 0.36, an unreasonable value, in pigs. In fact, if the average porcine small vessel hematocrit is assumed to be zero, small vessels would account for 24 percent of the circulating blood volume. In view of these calculations, it seems reasonable to conclude that porcine plasma volume was overestimated by the ^{125}I-dilution procedure used in this study. Alternatively, circulating erythrocyte volume may have been underestimated by the dilution of ^{51}Cr-labelled erythrocytes. This alternative seems highly unlikely since it would imply labelled-erythrocyte sequestration somewhere in the splenectomized pig. Decreasing label concentration following injection, which was not observed in this experiment, should have been seen if the labelled erythrocytes were so sequestered.

What could account for the overestimation of porcine plasma volume with the ^{125}I dilution procedure? Errors in isotope counting or sample volume measurement do not seem likely.

$$V_s = \frac{H_L - H_B}{H_L - H_S}$$

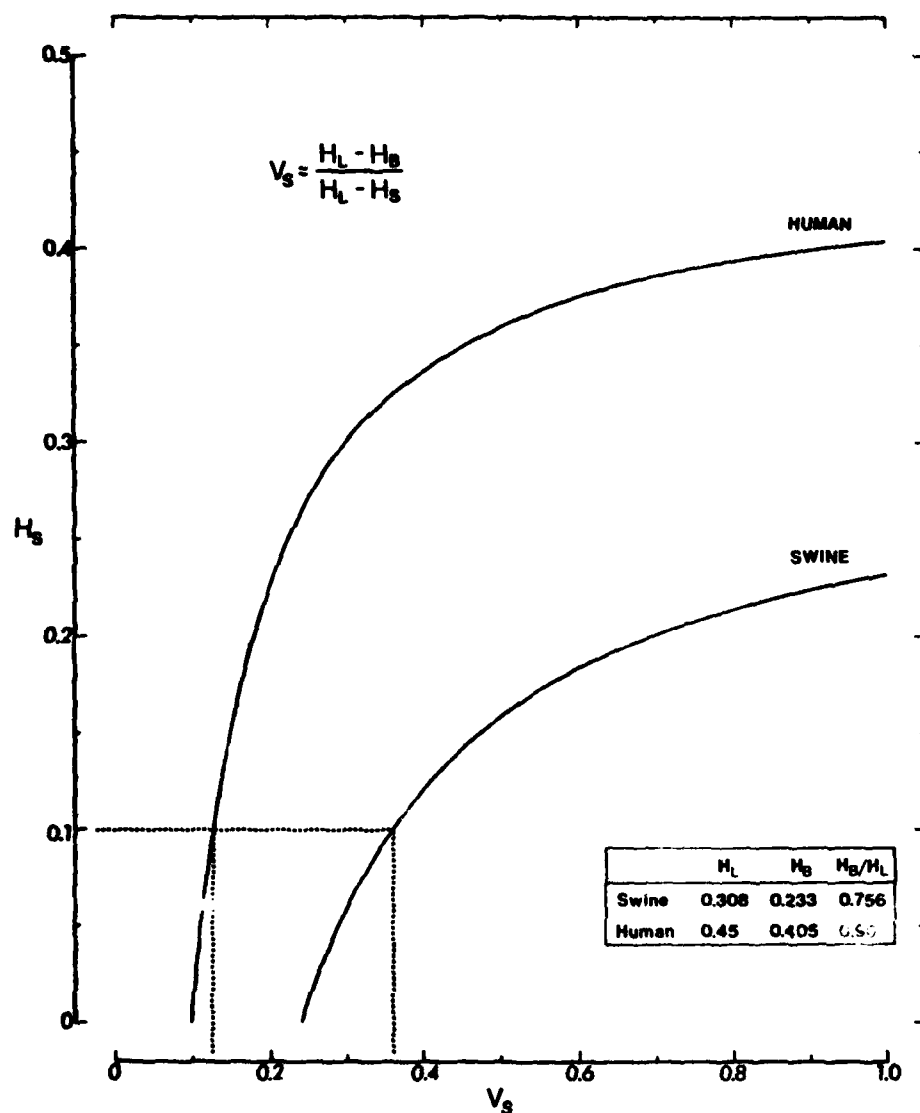|	H_L	H_B	H_B/H_L
Swine	0.308	0.233	0.756
Human	0.45	0.405	0.90

Figure 4.   Interrelationship of average small vessel hematocrit (H_s) to average small vessel fraction (V_s) of total blood volume as estimated by rearrangement of the equation [5].

Accuracy evaluations of both procedures showed potential errors should not exceed 1 percent. Furthermore, the same sampling pipettes and the same type of calibrated injection syringe were used in the ^{51}Cr and ^{125}I procedures. Potential errors, therefore, would have had an equal effect on both procedures. Contamination of radioiodine-labelled albumin with inorganic ^{125}I, which would rapidly leave the circulation following injection, could account for a small fraction of the overestimation of plasma volume. Determinations of the inorganic iodine fraction following precipitation of the albumin fraction with cold trichloroacetic acid or following separation with a molecular sieve showed that 95 percent or more of the ^{125}I was bound to albumin. Transcapillary loss of the inorganic fraction may have contributed to the early, more rapid decrease in plasma ^{125}I concentration seen here, but other investigators (34,35) have attributed this effect to delayed mixing of the albumin-labelled fraction. The most likely explanation for the overestimation of plasma volume would be a rapid early loss of radioiodinated albumin during the first minute or two after injection. Direct evidence for such a loss in dogs has been presented by Vidt and Saperstein (36), who concluded that circulating plasma includes a fraction which extends beyond the anatomical boundaries of capillary beds. The implications of, and support for, this concept in terms of microcirculatory hydrodynamics has been elegantly discussed by Howe and Sheaffer (37).

CONCLUSIONS

*   Under near-basal conditions the porcine spleen sequesters 20 to 25 percent of total body erythrocytes.

*   Splenically sequestered erythrocytes are rapidly discharged into the circulating blood following intravascular epinephrine injection or physical restraint of the animal. Both procedures lead to a marked increase in large vessel hematocrit.

*   Kinetic analysis of ^{51}Cr-labelled erythrocyte dilution following intravascular injection provides an accurate procedure for estimating circulating, total body, and splenic erythrocyte volumes. Simultaneous hematocrit measurements allow estimation of circulating plasma and blood volumes.

*   Plasma, erythrocyte, and blood volume estimates based on the dilution of ^{125}I-labelled bovine albumin markedly exceed those based on ^{51}Cr-labelled erythrocyte dilution.

*   Low values for the total body to large vessel ratio in pigs indicates overestimation of plasma volume by the ^{125}I-labelled albumin procedure.

RECOMMENDATIONS

* Experiments should be conducted to determine the factor(s) responsible for overestimation of porcine plasma volume when procedures based on the dilution of plasma albumin are used.

* The relationships of total body mass and lean body mass to circulating erythrocyte, plasma and blood volumes of swine should be investigated.

* Alterations, if any, in splenic erythrocyte storage capacity as a function of body size should be determined.

REFERENCES

1. Hansard SL, Sauberlich HE, Comar CL. Blood volume of swine. Proc Soc Exp Biol Med 1951;78:544-545.

2. Steinhardt M, Steinhardt K, Wachtel W, Lyhs L. Untersuchungen über das Plasmavolumen, Blutvolumen and die totale Hämoglobinmenge bei Schweinen. Arch Exp Ved Med 1972;26:533-550.

3. von Engelhardt W. Swine cardiovascular physiology--a review. In: Bustad LK, McClellan RO, eds. Swine in biomedical research. Seattle, WA: Frayn Printing, 1966:307-329.

4. Doornenbal H, Asdell SA, Wellington GH. Chromium-51 determined red cell volume as an index of "lean body mass" in pigs. J Anim Sci 1962;21:461-463.

5. Doornenbal H, Martin AH. The evaluation of blood volume and total red cell mass as predictors of gross body composition in the pig. Can J Anim Sci 1965;45:203-210.

6. Schuttert R, Steenberg K, Standal N, Lien H. Blut volumen--und Ultraschallmessungen an lebenden Schlactschweinen als Ausdruck der Fleisch--und Fettfulle ihrer Schlachtkorper. Acta Agric Scand 1968;18:101-108.

7. Wachtel TL, McCahan GR Jr. The contractile response of the spleen of miniature swine to intra-arterial infusion of epinephrine. J Anim Sci 1974;24:329-334.

8. Steinhardt M, Petzold K, Lyhs L. Blutspeicherfunktion der Milz beim Hausschwein. I. Einfluss von Adrenalin und korperlicher Arbeit auf den Hämatokritwert und Hämoglobingehalt. Arch Exp Vetmed 1970;24:817-826.

9. Steinhardt M, Petzold K. Lyhs L. Blutspeicherfunktion der Milz beim Hausschwein. II. Einfluss von Adrenalin und einer Laufarbeit auf Plasma--und Blutvolumen. Arch Exp Vetmed 1970;24:827-839.

10. Steinhardt M, Steinhardt K. Einfluss der Umgebungstemperatur auf die Veränderungen des Blut--und Plasmavolumens durch Laufarbeit bie Hausschweinen ohne Milz. 1971;25:997-1005.

11. Steinhardt K, Steinhardt M, Lyhs L. Einfluss der Umgebungstemperatur auf die Veranderungen des Blut--und Plasmavolumens durch Laufarbeit beim Hausschwein. Arch Exp Vetmed 1971;25:363-369.

12. Reeve EB, Gregersen MI, Allen 'M, Sear H. Distribution of cells and plasma in the normal and splenectomized dog and its influence on blood volume estimates with P^{32} an T-1824. Am J Physiol 1953;175:195-203.

13. Nelson AW, Swan H. The effects of the canine spleen on red cell and blood volume determinations using ^{51}RBC [sic]. Surgery 1972;71:215-224.

14. Baker CH. Cr^{51}-labeled red cell, I^{131}-fibrinogen and T-1824 dilution spaces. Am J Physiol 1963;204:176-180.

15. Tumbleson ME, Kalish PR. Serum biochemical and hematological parameters in cross-bred swine from birth through eight weeks of age. Can J Comp Med 1972;36:202-209.

16. Copland JW. Normal haematological parameters of pigs in Papua, New Guinea. Trop Anim Health Prod 1976;8:63-69.

17. Miller ER, Ullrey DE, Ackermann I, Schmidt DA, Luecke RW, Hoefer JA. Swine hematology from birth to maturity. II. Erythrocyte population, size, and hemoglobin concentration. J Anim Sci 1961;20:890-897.

18. Nasi M, Saloniemi H, Kallela K. Hematological and blood chemical data of pigs fed various amounts of mink manure. Acta Vet Scand 1978;19:84-92.

19. Finsterer U, Prucksunand P, Brechtelsbauer H. The role of the spleen in determination of red cell volume with Cr^{51}-labelled erythrocytes in the intact dog. Pflügers Arch 1973;341:73-76.

20. Breznock EM, Strack D. Effects of the spleen, epinephrine, and splenectomy on determination of blood volume in cats. Am J Vet Res 1982;43:2062-2066.

21. Hansard SL. Residual organ blood volume of cattle, sheep, and swine. Proc Soc Exp Biol Med 1956;91:31-34.

22. Hansard SL, Butler WO, Comar CL, Hobbs CS. Blood volume of farm animals. J Anim Sci 1963;22:402-413.

23. Bush JA, Jensen WN, Cartwright GE, Wintrobe MM. Blood volume studies in normal and anemic swine. Am J Physiol 1963;181:9-14.

24. Talbot RB, Swenson MJ. Blood volume of pigs from birth through 6 weeks of age. Am J Physiol 1970;218:1141-1144.

25. Setiabudi M, Sheng H-P, Huggins RA. Growth of the pig: changes in red cell and plasma volumes. Growth 1976;40:127-132.

26. Deavers SI, Huggins RA, Sheng H-P. Blood volume changes during the first week after birth in the beagle and pig. Proc Soc Exp Biol Med 1978;159:152-156.

27. Linderkamp O, Berg D, Betke K, Koferl F, Friegel H, Riegel KP. Blood volume and hematocrit in various organs in newbon piglets. Pediat Res 1980;14:1324-1327.

28. Talbot RB, Swenson MJ. Measurement of porcine plasma volume, using T-1824 dye. Am J Vet Res 1963;24:467-471.

29. Sybesma W, Hart PC. Bloedvolume en vleesdegenerate bij varkens. Tijdschr Diergeneesk 1965;90:1116-1121.

30. Ramirez CG, Miller ER, Ullrey DE, Hoefer JA. Swine hematology from birth to maturity. III. Blood volume of the nursing pig. J Anim Sci 1963;22:1068-1074.

31. Pownall R, Dalton RG. Blood and plasma volumes of neonatal pigs expressed relative to body weight and total body water. Brit Vet J 1973;129:583-587.

32. Chaplin H Jr, Mollison PL, Vetter H. The body/venous hematocrit ratio: its constancy over a wide hematocrit range. J Clin Invest 1953;32:1309-1316.

33. Lipowski HH, Usami S, Chien S. In vivo measurments of "apparent viscosity" and microvessel hematocrit in the mesentery of the cat. Microvasc Res 1980;19:297-319.

34. Baker CH, Wykoff HD. Time concentration curves and dilution spaces of T-1824 and I^{131}-labelled proteins in dogs. Am J Physiol 1961;201:1159-1163.

35. Swan H, Nelson AW. Blood volume I: Critique: Spun vs. isotope hematocrit; ^{125}RIHSA vs. ^{51}CrRBC. Ann Surg 1971;173:481-495.

36. Vidt DG, Saperstein LA. Distribution volumes of T-1824 and chromium^{51} labelled red cells immediately following intravenous injection. Circ Res 1957;5:129-132.

37. Howe JT, Shaeffer YS. Analysis of a recent hypothesis of plasma flow in pericapillary space. Circ Res 1967;21:925-934.

OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
 and Development Command
ATTN: SGRD-RMS/Mrs. Madigan
Fort Detrick, Frederick MD 21701

Defense Technical Information Center
ATTN: DTIC-DDA   (12 copies)
Cameron Station
Alexandria VA 22314

Director of Defense Research and Engineering
ATTN: Assistant Director, Environmental
 and Life Sciences
Washington DC 20301

The Surgeon General
ATTN: DASG-TLO
Washington DC 20314

HQ DA (DASG-ZXA)
WASH DC 20310

Commandant
Academy of Health Sciences
ATTN: HSHA-CDM
Fort Sam Houston TX 78234

Assistant Dean
Institute and Research Support
Uniformed Services University
 of Health Sciences
6917 Arlington Road
Bethesda MD 20014

Commander
US Army Environmental Hygiene Agency
Aberdeen Proving Ground MD 21070

US Army Research Office
ATTN: Chemical and Biological Sciences
 Division
P.O. Box 1221
Research Triangle Park NC 27709

Biological Sciences Division
Office of Naval Research
Arlington VA 22217

Director of Life Sciences
USAF Office of Scientific Research (AFSC)
Bolling AFB
Washington DC 20332

Director
Walter Reed Army Institute of Research
Washington DC 20307

Commander
US Army Medical Research Institute
 of Infectious Diseases
Fort Detrick, Frederick MD 21701

Commander
US Army Research Institute
 of Environmental Medicine
Natick MA 01760

Commander
US Army Institute of Surgical Research
Brooke Army Medical Center
Fort Sam Houston TX 78234

Commander
US Army Medical Bioengineering
 Research and Development Laboratory
Fort Detrick, Frederick MD 21701

Commander
US Army Aeromedical Research Laboratory
Fort Rucker AL 36362

Commander
US Army Research Institute
 of Chemical Defense
Aberdeen Proving Ground
Edgewood Arsenal MD 21010

Commander
Naval Medical Research Institute
National Naval Medical Center
Bethesda MD 20014

Commander
USAF School of Aerospace Medicine
Aerospace Medical Division
Brooks Air Force Base TX 78235